(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
21 November 2002 (21.11.2002)

PCT

(10) International Publication Number
WO 02/093168 A1

(51) International Patent Classification[7]: G01N 33/53, 33/68, 27/447, B01D 57/02

(21) International Application Number: PCT/AU02/00592

(22) International Filing Date: 15 May 2002 (15.05.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

PR 5015	15 May 2001 (15.05.2001)	AU
PR 6100	4 July 2001 (04.07.2001)	AU

(71) Applicant: GRADIPORE LIMITED [AU/AU]; 22 Rodborough Road, Frenchs Forest, NSW 2086 (AU).

(72) Inventors: WANG, Kailing; 21 Mulyan Avenue, carlingford, NSW 2188 (US). NAIR, Chenicheri, Hariharan; 80 Tomac Avenue, Old Greenwich, CT 06870 (US).

(74) Agent: ALLENS ARTHUR ROBINSON PATENT & TRADE MARKS ATTORNEYS; Chifley Tower, 2 Chifley Square, Sydney, NSW 2000 (AU).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/093168 A1

(54) Title: PRION DIAGNOSTIC TEST

(57) Abstract: The invention relates to a method for detecting prions in a sample, the method comprising (a) subjecting a sample suspected of containing prions to membrane-based electrophoresis to separate and/or concentrate at least some prions present in the sample; and (b) detecting the presence of the separated or concentrated prions.

PRION DIAGNOSTIC TEST

Technical Field

The present invention relates to tests for the detection of infectious agents, particularly an assay for the detection of prions in samples using membrane-based electrophoresis.

Background Art

Bovine spongiform encepalopathy (BSE), scrapie of sheep, Kuru and Creutzfeldt-Jakob disease (CJD) of humans are only a few examples of a group of neurodegenerative disorders named transmissible spongiform encepalopathies (TSE) which are characterised by loss of motor control, dementia, paralysis, blindness, wasting and eventually death. These diseases may be inherited or sporadic. The primary health risk of TSE for humans is believed to be through food products derived from BSE-infected cattle. A further transmission risk is a possible infection through human blood and blood products which originated from TSE-infected donors.

Recently, it was shown that these fatal neurodegenerative diseases are caused by a newly discovered infectious pathogen named prion protein (PrP) (Prusiner S.B., *P.N.A.S.* **95**, 13363-13383, 1998). More precisely, it is the accumulation of the infectious isoform of the PrP into amyloid plaques which result in the development of the disease. The different isoforms of PrP have been identified as the normal cellular form (PrP^{c}) and the highly infectious scrapie form (PrP^{Sc}). The PrP^{Sc} form of the protein was found to be protease and detergent resistant, while PrP^{c} has been shown to be sensitive to the conventional treatment processes causing protein degradation and denaturation. Although identical in amino acid sequence, the two proteins have been shown to have different conformational characteristics with PrP^{c} containing more α-helical structure than its infectious counterpart. Nevertheless, there has been no effective method of discriminating between the two proteins by the way of immunoreagents. Only recently, plasminogen was recognised as the first

naturally occurring PrP^{Sc}-binding protein that can distinguish between PrP^{c} and PrP^{Sc} (Fischer M.B., *Nature*, **406**, 479-483, 2000).

With the incubation time in humans of up to 40 years, early diagnosis of any of these fatal neurodegenerative diseases should limit the potential of their further transmission and possibly assist in treatment of disease. Currently, there are no commercial non-invasive assays that allow detection of prions in infected individuals prior to the development of clinical symptoms of disease. The few tests that are in use are able to detect prions in brain tissue and the spinal cord only and are largely used on animals and humans in their post-mortem state. One of the major challenges of the "mad cow disease" epidemic is the provision of a sensitive and reliable diagnostic test to identify people and animals that have been infected with the infectious form of PrP before they become symptomatic. Blood is the preferred choice for analysis in any diagnostic assay as it is accessible by venesection, therefore less invasive than other biological materials.

In the preclinical phase of prion infection in experimentally infected rodents, it was identified that PrP^{Sc} infectivity was closely associated with blood buffy coat, leucocyte, platelet and plasma fractions. While blood is a good candidate for a diagnostic test, other tissue samples or fluids from the living organism can be used.

The present inventors have now developed a test for prions based on membrane-based electrophoresis separation.

Disclosure of Invention

In a first aspect, the present invention provides a method for detecting prions in a sample, the method comprising:-

(a) subjecting a sample suspected of containing prions to membrane-based electrophoresis to separate and/or concentrate at least some prions present in the sample; and

(b) detecting the presence of the separated or concentrated prions.

The sample can be any liquid sample suitable for undergoing electrophoresis. Preferably, the sample is a biological sample including blood,

plasma, serum, blood, cell products or extracts, cerebrospinal fluid (CSF), tissue homogenates, urine, semen or combinations thereof.

In one preferred form, step (a) comprises:

subjecting a sample suspected of containing prions to membrane-based electrophoresis to separate and concentrate at least some prions into a smaller volume.

In one preferred form, a small electrophoresis apparatus having static chambers is used for step (a). In this form, step (a) includes

(i) applying a sample to a sample chamber of an electrophoresis apparatus adapted to receive small volumes comprising a static cathode compartment, a static anode compartment, an electrophoresis separation membrane having a defined pore size and pore size distribution disposed between the anode and cathode compartments, a first restriction membrane disposed between the cathode compartment and the separation membrane so as to define a sample chamber having a small interstitial volume, and a second restriction membrane disposed between the anode compartment and the separation membrane so as to define a separation chamber having a small interstitial volume, wherein in use there is substantially no recirculation of liquid in the compartments or chambers; and

(ii) applying an electric potential to the sample in the sample chamber such that prions in the sample move toward the separation membrane and preferably pass through the membrane into the separation chamber; or

(iii) applying an electric potential to the sample in the sample chamber such that components other than prions in the sample move toward the separation membrane and preferably pass through the membrane into the separation chamber.

Preferably, the small apparatus further comprises:

cathode positioned in the cathode compartment and anode positioned in the anode compartment.

The apparatus can have an alternate configuration where the anode and cathode compartments are reversed. In this form, the sample chamber is disposed adjacent the anode compartment and the separation chamber is disposed adjacent the cathode compartment. One means of obtaining this alternative configuration is to reverse the polarity of the electrodes.

To contain prions in the sample and/or separation chambers, the restriction membranes preferably do not allow substantial movement of prions to the respective electrode compartments.

Preferably, the small volume is less than about 10 mL, preferably about 5 mL or less. The static fluid apparatus is particularly suitable for separating samples of about 1 to about 0.02 mL. Larger volumes can be processed depending on the configuration of the electrophoresis apparatus used.

A ratio of sample to separation membrane surface area of less that about 5 mL/cm^2 or even 1 mL/cm^2 is required. Preferably, the ratio is 0.5 mL/cm^2 or less, more preferably the ratio is 0.1 mL/cm^2 or less, and more preferably around 0.02 mL/cm^2.

The method according to the present invention practiced using a small electrophoresis apparatus is suitable for testing a number of samples, preferably in arrays using multi-well plates. The method could ultimately be automated by using an electrophoresis apparatus capable of running a number of samples which would be deposited on multi-well plates (pre-coated with a selective PrP-binding agent) for further prion concentration or detection.

Examples of small electrophoresis apparatus suitable for use in the present invention can be found in WO 01/78878, incorporated herein by reference.

In another preferred form, step (a) is carried out using an electrophoresis apparatus comprising:-

(i) an electrolyte reservoir;

(ii) a first sample reservoir and a second sample reservoir;

(iii) a separation unit having a first electrolyte chamber in fluid connection with the electrolyte reservoir, a second electrolyte chamber in fluid connection with the electrolyte reservoir, a first sample chamber positioned between the first electrolyte chamber and the second electrolyte chamber, a second sample chamber positioned adjacent to the first sample chamber and between the first electrolyte chamber and the second electrolyte chamber, the first sample chamber being in fluid connection with the first sample reservoir, and the second sample chamber being in fluid connection with the second sample reservoir;

(iv) a first ion-permeable barrier positioned between the first sample chamber and the second sample chamber, the first ion-permeable barrier

prevents substantial convective mixing of contents of the first and second sample chambers;

(v) a second ion-permeable barrier positioned between the first electrolyte chamber and the first sample chamber, the second ion-permeable barrier prevents substantial convective mixing of contents of the first electrolyte chamber and the first sample chamber;

(vi) a third ion-permeable barrier positioned between the second sample chamber and the second electrolyte chamber, the third ion-permeable barrier prevents substantial convective mixing of contents of the second electrolyte chamber and the second sample chamber;

(vii) electrodes positioned in the first and second electrolyte chambers;

(viii) means for supplying electrolyte from the electrolyte reservoir to the first electrolyte chamber and the second electrolyte chamber; and

(ix) means for supplying sample or liquid from at least the first sample reservoir to the first sample chamber, or from the second sample reservoir to the second sample chamber.

In one form, the first ion-permeable barrier is a membrane having a characteristic average pore size and pore size distribution. In one form, all the ion-permeable barriers are membranes having a characteristic average pore size and pore size distribution. This configuration of the apparatus is suitable for separating on the basis of charge and or size.

The apparatus may further comprise:

(x) means for circulating contents from each of the first and second sample reservoirs through the respective first and second sample chambers forming first and second sample streams in the respective sample chambers.

Preferably, means (x) is a pump arrangement controllable for movement of the sample streams.

The apparatus may further include:

(xi) means for removing and replacing sample in the first or second sample reservoirs.

The apparatus may also further include:

(xii) means to maintain temperature of electrolyte and sample solutions.

In another form, the separation unit is provided as a cartridge or cassette housing the ion-permeable barriers fluidly connected to the electrolyte reservoirs and the sample reservoirs.

In use, a sample to be tested is placed in the first and/or second sample reservoirs and provided to, or circulated through, the first and/or second chambers. Electrolyte is placed in the electrolyte reservoir and passed to, or circulated through, the first and second electrolyte chambers. Electrolyte or other liquid can be placed in the first and/or second sample reservoirs if required. An electric potential is applied to the electrodes wherein one or more components in the first and/or second sample chamber are caused to move through a barrier to the second and/or first sample chamber, or to the first and/or second reservoir chambers. Treated sample or components separated from the sample can be collected in the second and/or first sample reservoir.

In use, the cathode zone and the anode zone are supplied with suitable buffer solutions by any suitable pumping means. A sample to be processed is supplied directly to the first or second interstitial volumes by any suitable pumping means.

Preferably, the zones and the first and second streams are configured to allow flow of the respective fluid/buffer and sample solutions forming streams. In this form, larger volumes can be processed quickly and efficiently. The solutions are typically moved or recirculated through the zones and streams from respective reservoirs by suitable pumping means. In a preferred embodiment, peristaltic pumps are used as the pumping means for moving the sample, buffers or fluids.

In one embodiment, the buffer and sample solutions are cooled by any suitable means to ensure no inactivation of sample constituents occurs during the separation process and to maintained a desired temperature of the apparatus while in use.

Preferably, in order to collect and/or concentrate separated constituents, solution in at least one of the streams containing any separated compounds or molecules is collected and replaced with suitable solvent to ensure that electrophoresis can continue in an efficient manner.

It will be appreciated that this procedure can be reversed where a sample is placed in the second solvent stream, the polarity of the electrodes being reversed so the product is collected in the first solvent stream. In some applications, the sample can be placed in either the first or second solvent stream and the prions moved to the opposite stream. Alternatively, the prions are caused to remain in the original stream while other components are removed from the sample into the other solvent stream.

The first ion-permeable barrier is preferably an electrophoresis separation membrane which is preferably comprised of polyacrylamide and having a defined molecular mass cut-off. Preferably, the electrophoresis separation membrane has a molecular mass cut-off up to about 1500 kDa. It will be appreciated, however, that other membrane chemistries or constituents can be used.

A molecular mass cut off of about 1000 kDa has been found to be particularly suitable for the electrophoresis separation membrane. It will be appreciated, however, that other cut-off membranes that allow the movement of prions or other components in samples being processed would also be suitable.

The second and third ion-permeable barriers are preferably restriction membranes which are preferably formed from polyacrylamide and have a molecular mass cut-off less than the separation membrane, preferably from about 1 kDa to about 500 kDa. The molecular mass cut-off of the restriction membrane will depend on the sample being processed and the size of the components to be removed or retained.

The molecular mass cut-off of the restriction membranes are usually less than the molecular mass cut-off of the separation membrane.

In a preferred form, at least one membrane forming the barrier is capable of controlling substantial bulk movement of liquid under the influence of an electric field an inducible electro-endo-osmotic membrane. The inducible electro-endo-osmotic membrane is preferably a poly-vinyl alcohol (PVA) membrane. It will be appreciated that the inducible electro-endo-osmotic membrane can be formed from any other suitable membrane material such as cellulose tri-acetate (CTA).

The present inventors have found that a PVA membrane having a nominal molecular mass cut-off of 22 kDa is particularly suitable for use in the apparatus

according to the present invention. It will be appreciated that other molecular mass cut-offs would also be suitable for the present invention.

An electrophoresis separation membrane of about 1000 kDa and two restriction membranes of about 10 kDa have been found to be particularly suitable for prion processing according to the present invention. Depending on the pH of the sample and electrophoresis buffer, the prions can be negatively charged, positively charged or have no charge. Movement of prions (and other compounds in a sample) by electrophoresis will depend on the charge and size of the prions and/or any other compounds in the sample to be processed. By selecting the buffer and molecular mass cut off of the separation and restriction membranes, various separations of prions and other compounds in a given sample can be devised.

Voltage and/or current applied can vary depending on the separation. Typically up to many hundred volts may be used but choice and variation of voltage will depend on the configuration of the apparatus, buffers and the sample to be separated or treated. In a laboratory scale instrument, the preferred voltage is about 250 V.

As prions are usually present in very low concentrations in samples and are therefore extremely difficult to detect, step (a) is preferably used to concentrate prions in the sample or the separation chamber by removing other components or selectively separating the prions from the sample or by transferring prions from a large volume to a smaller volume.

Preferably, a selective agent which binds infectious prions (PrP^{Sc}) and not normal prions (PrP^{c}) is used in step (b). In this mode, both PrP^{c} and PrP^{Sc} will be recovered by the electrophoresis step (a) and detection and discrimination between PrP^{c} and PrP^{Sc} is made by using the selective binding agent in step (b).

The agent can be a biological agent which selectively binds prions, preferably infectious prions or a chemical agent that prions, preferably selectively binds infectious prions. Preferably, the biological agent is selected from proteins, peptides, small molecules, antibodies, binding fragments of antibodies, plasma components, plasminogen, angiostatin, fibrin, fibrinogen or combinations thereof. Preferably the chemical agent is a heteropoly acid or salts thereof. The

heteropoly acid is preferably phosphotungstic acid or salts thereof as disclosed in US 6166187.

Two types of specific PrP^{Sc} antibodies are currently available. One type of antibody is the conformation-specific antibodies (Yokoyama et al. Journal of Biological Chemistry, 276(14), 11265-11271); the other type is the epitope-specific antibody described in an US patent (O'Rourke et al, 6165784). These antibodies would be suitable for use in step (b) of the present invention.

In one preferred embodiment, the method further includes:

(c) detecting the presence of infectious prions PrP^{Sc} if is present in the samples or distinguishing PrP^{Sc} from PrP^{c}.

In an other preferred embodiment, the method further includes:-

(d) characterising the detected prions, preferably characterising infectious prions. The characterising can be in the form of solubility, three-dimensional structure, Circular Dichroism (CD), hydrophobicity or other physical and biochemical properties and infectivity for example.

In a second aspect, the present invention provides a kit for detecting prions in a sample, the kit comprising:-

(a) a membrane-based electrophoresis apparatus suitable for separating and/or concentrating prions from samples; and

(b) means for detecting prions separated and/or concentrated by the electrophoresis apparatus.

The sample can be any liquid sample suitable for undergoing electrophoresis. Preferably, the sample is a biological sample including blood, plasma, serum, blood, cell products or extracts, cerebrospinal fluid (CSF), tissue homogenates, urine, semen or combinations thereof.

Preferably, means (b) is a prion capture system utilising a selective binding agent. The agent can be a biological agent which selectively binds prions, preferably infectious prions, or a chemical agent that binds prions, preferably selectively binds infectious prions. The biological agent can be a protein, peptide or other small molecule, antibody, binding fragment of an antibody. Preferably,

the biological agent is plasminogen, angiostatin, fibrin, fibrinogen or antibody. Preferably the chemical agent is a heteropoly acid or salts thereof. The heteropoly acid is preferably phosphotungstic acid or salts thereof. The means can be an immuno-detection system including Western Blot, ELISA, time-resolved dissociation-enhanced fluoroimmunoassay (DELFIA), or radioimmunoassay.

Preferably, means (b) allows detection and identification of infectious prions.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is solely for the purpose of providing a context for the present invention. It is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed in Australia before the priority date of each claim of this application.

In order that the present invention may be more clearly understood, preferred forms will be described with reference to the following drawings and examples.

Brief Description of the Drawings

Figure 1 shows the separation of bovine PrP^c from bovine brain homogenate. Separation of PrP^c from bovine brain homogenate was carried out for 3 hours at 250V using a cartridge with a separation membrane of 150 kD and two restriction membranes of 10 kDa. When 20 mM Tris Borate buffer (pH 9.0) was used during electrophoresis, PrP^c remained in the first sample chamber (see Panel A). By changing the electrophoresis conditions (eg. reverse polarity, 30 mM GABA/Acetic Acid buffer (pH 4.6)), PrP^c was completely transferred to the

second sample chamber under same membrane configurations and voltage conditions (Panel B). $S1_0$ is Stream 1 at time 0 minutes, $S1_{180}$ is Stream 1 at time 180 minutes, $S2_0$ is Stream 2 at time 0 minutes, $S2_{180}$ is Stream 2 at time 180 minutes. The results show that electrophoresis can be used to successfully separate or retain prions from or in samples. The electrophoresis step allows the capture and partial purification of prions.

Figure 2 shows a Western blot using the anti-plasminogen antibody to probe anti-PrP immunoprecipitates. A mixture of human plasma and bovine brain homogenate (1:3 v/v) was incubated with anti-PrP antibody at room temperature for 2 hours to allow plasminogen/PrP immunocomplex to form. This was followed by the addition of Protein A and Protein G agarose, respectively for another two-hour incubation at room temperature which would allow for Protein A or Protein G to immunoprecipitate the plasminogen/PrP immunocomplex formed. Plasminogen present in the anti-PrP immunoprecipitates was detected by Western Blot using anti-Plasminogen antibody.

Figure 3 shows SDS-PAGE analysis of electrophoresis run number 1 & 7 as shown in Table 1. Lanes $S1_0$ is Stream 1 at time 0 minutes, $S1_{120}$ is Stream 1 at time 120 minutes, $S2_0$ is Stream 2 at time 0 minutes, $S2_{120}$ is Stream 2 at time 120 minutesof run 1, respectively. Lanes $S1_0$ is Stream 1 at time 0 minutes, $S1_{120}$ is Stream 1 at time 120 minutes, $S2_0$ is Stream 2 at time 0 minutes, $S2_{120}$ is Stream 2 at time 120 minutes of run 7, respectively.

Figure 4 shows SDS PAGE analysis of charge-based separation using a Large-type separation membrane having a molecular mass cut-off of 1000 kDa (step 1), followed by a size-based separation using Small-type separation membrane having a molecular mass cut-off of 200 kDa (step 2).

Figure 5 shows SDS PAGE analysis of charge-based separation using a Large-type separation membrane having a molecular mass cut-off of 1000 kDa (step 1), followed by a size-based separation using Small-type separation membrane having a molecular mass cut-off of 200 kDa and a CTA membrane as an upper restriction membrane (step 2).

Figure 6 shows analysis of simultaneous separation and concentration of the bovine prion protein (PrP) from bovine brain homogenate using membrane-

based electrophoresis technology and the PVA membrane. Panel A: SDS-PAGE of the samples from the electrophoresis run; Panel B: Western blot of the samples from the electrophoresis run using anti-PrP R029 (Prionics, Switzerland); $S1_0$ Stream 1 at time 0 minutes; $S1_{180}$ Stream 1 at time 180 minutes; $S2_0$ Stream 2 at time 0 minutes; $S2_{180}$ Stream 2 at time 180 minutes. Separation of PrP was carried out for 3 hours at 250V using a cartridge with a separation membrane of 200 kDa, and two restriction membranes of 10 kDa with Tris Borate buffer (20 mM Boric Acid, 45 mM Trizma Base, pH 9.0) being applied (Panel A, lanes 1-4), the conditions under which PrP^C remained in the upstream (Panel B, lanes 1-4). Simultaneous separation and concentration of PrP^C from bovine brain homogenate was achieved under the same buffer running conditions, but with a PVA membrane being used as the top restriction membrane in place of the 10kDa membrane (Panel B, lanes 5-8). There was a noticeable increase in the concentration of PrP, as detected in the increase of the intensity of the PrP band on the Western blot (panel B).

Mode(s) for Carrying Out the Invention

METHODS

Apparatus

A number of membrane-based electrophoresis apparatus developed by Gradipore Limited, Australia were used in the following experiments. In summary, the apparatus typically includes a cartridge which houses a number of membranes forming two chambers, cathode and anode connected to a suitable power supply, reservoirs for samples, buffers and electrolytes, pumps for passing samples, buffers and electrolytes, and cooling means to maintain samples, buffers and electrolytes at a required temperature during electrophoresis.

The cartridge contained three substantially planar membranes positioned and spaced relative to each other to form two chambers through which sample or solvent can be passed. A separation membrane is positioned between two outer membranes (termed restriction membranes as their molecular mass cut-offs are usually smaller than the cut off of the separation membrane). When the cartridge is installed in the apparatus, the restriction membranes are located adjacent to an

electrode. The cartridge is described in AU 738361, which description is incorporated herein by reference.

Description of membrane-based electrophoresis can be found in US 5039386 and US 5650055 in the name of Gradipore Limited, which description is incorporated herein by reference.

Samples

Normal prion protein (PrPc) samples from either bovine brain tissue homogenates or human plasma were used in all experiments. As infectious prion protein (PrP^{Sc}) is difficult to obtain and work with, PrPc samples were used to demonstrate that the present invention has utility. It will be appreciated that the results obtained with PrPc should be applicable to samples containing PrP^{Sc}.

Separation conditions

Using human plasma as a starting material, size-based separation of normal prion protein (PrPc) was performed followed by a charge-based separation. The amount of prion protein in human plasma was quantified by a time-resolved dissociation-enhanced fluoroimmunoassay DELFIA (EG&G Wallac). All samples generated from the electrophoresis runs were analysed by SDS-PAGE for protein separation. Based on the characteristics of human plasma prion protein, a 2-step separation process was developed to remove plasma protein contaminants and to purify prion protein. This was followed with a simultaneous separation and concentration of human plasma PrP by utilising CTA EEO membranes. PrPc present in these samples were quantified by DELFIA assay (EG&G Wallac).

PAGE analysis of membrane-based separations

A two step separation process of PrPc was performed in order to achieve the best separation of PrPc from the majority of normal human plasma proteins.

First step separation (a charge-based separation): Charged-based separation of PrPc was carried out in reverse polarity for 2 hours at 250V using MOPS/GABA buffer (80mM MOPS, 20mM GABA, pH 5.0) as the running buffer. The cartridge set up consisted of two small-type restriction membranes with 10

kDa molecular weight cut off and a large-type separation membrane with 1000 kDa molecular weight cut off. Both high and low molecular weight proteins with pIs greater than 5.0 were removed to S2 and the partially purified PrPc remained in S1. The PrP fraction from the first step was then used as the starting material for the second step.

Second step separation (a size-based separation): Size-based separation of PrPc was carried out in forward polarity for 2 hours at 250V using Tris Borate buffer (20mM Boric Acid, 45mM Trizma Base, pH 9.0) as the running buffer. The cartridge set up consisted of two small-type restriction membranes with 10 kDa molecular weight cut off and a small-type separation membrane with 200 kDa molecular weight cut off. Low molecular weight proteins with pIs less than 9.0 were removed to S2 while PrPc was fully retained in S2.

Two-step separation protocol resulted in removal of contaminant proteins with pI between 5 to 9 (90% Albumin, 80% IgG, 100% a1-PI removed) as shown by SDS-PAGE.

RESULTS

Separation of PrP^c from bovine brain homogenate

Separation of PrP^c from bovine brain homogenate was carried out for 3 hours at 250V using a cartridge with a separation membrane of 150 kD and two restriction membranes of 10 kDa. When 20 mM Tris Borate buffer (pH 9.0) was used during electrophoresis, PrP^c remained in the sample chamber (see Panel A of Figure 1). By changing the electrophoresis conditions (eg. reverse polarity), PrP^c was completely transferred to the separation chamber under same membrane configurations and voltage conditions but with 30 mM GABA/Acetic Acid buffer (pH 4.6) (Panel B of Figure 1). The results show that electrophoresis can be used to successfully separate or retain prions from or in samples. The electrophoresis step allows the capture and partial purification of prions.

A mixture of human plasma and bovine brain homogenate (1:3 v/v) was incubated with anti-PrP antibody at room temperature for 2 hours to allow plasminogen/PrP immunocomplex to form. This was followed by the addition of Protein A and Protein G agarose, respectively for another two-hour incubation at room temperature which would allow for Protein A or Protein G to

immunoprecipitate the plasminogen/PrP immunocomplex formed. The plasminogen present in anti-PrP immunoprecipitates was then detected on a Western blot using the anti-plasminogen antibody (Figure 2).

Experimental results showed that this electrophoresis method can separate bovine prion protein from bovine brain tissue homogenates. As shown in Figure 1 (Panel A and B), the bovine prion protein can either be completely transferred from the sample material in the first stream to the second stream or retained in the first stream after electrophoresis separation. In addition, immunoaffinity experiments showed that that there is a naturally occurring interaction between plasminogen and prion protein (Figure 2). These results show that the present invention has the potential capability of separating and concentrating the prion protein from blood fractions.

Using a PrP^{Sc}-selective binding agent (such as plasminogen, anti-PrP^{Sc} antibody, angiostatin, fibrin, fibrinogen or chemical agent) chemically-immobilised on a support matrix, eg. Agarose beads or 96 well plates, PrP^{Sc} in the sample fractions following separation can be captured and concentrated by the immobilised matrix and subsequently detected by Western Blot or ELISA. Therefore a diagnostic assay can be developed to detect prions and prion infectivity in blood or other biological samples.

In a first step, electrophoresis separation is used to at least partially separate PrP from blood fractions such as plasma and platelets. A plasminogen, angiostatin, fibrin, fibrinogen or PrP^{Sc}-specific antibody-immobilised matrix can then used to identify and concentrate the PrP^{Sc} in the treated sample after electrophoresis separation. PrP^{Sc} can then be treated with proteinase K for example and subsequently detected by Western Blot using a specific anti-prion antibody.

Detection of prions in whole blood would be a good candidate for preclinical diagnosis as it is probable that prions would circulate in small amounts in infected individuals.

An electrophoresis separation apparatus can be modified so that the samples from the separation chamber can flow or be deposited to a 96-well PrP^{Sc} selective binding agent (eg. plasminogen, angiostatin, fibrin, fibrinogen or PrP^{Sc}-

specific antibody)-immobilised plates. Following the separation, PrP^{Sc} can be detected by ELISA using a specific anti-prion antibody or by radioimmunoassy for example.

Alternatively, plasminogen (angiostatin, fibrin, fibrinogen or another macro or micromolecule or particle or PrP^{Sc}-specific antibody) is coated to the separation membrane before plasma or platelet samples are subjected to electrophoretic separation. Prion protein remaining in samples can be analysed by Western Blot or ELISA analysis after its subsequent dissociation from the membrane by addition of denaturing reagent.

From a review of the literature, about eight different blood-screening test methods are in different stages of development. Several methods appear to have threshold PrP detection sensitivities within the range of what might be encountered in the blood of infected individuals, but none has yet been shown to be capable of detecting PrP^{Sc} in the blood of human beings who are incubating CJD (Brown *et al.* 2001 Blood infectivity and prospects for a diagnostic screening test in Creutzfeldt-Jakob disease. *J. Lab. Clinic. Med.* **137 (1),** 5-13, 2001). In addition, all the methods and instruments used for detection have two critical variables: inherent sensitivity of the method and specimen volume tested by the instrument. A distinct advantage of the present invention is that an electrophoresis apparatus can perform prion separation with a small volume of blood fractions. Subsequently PrP^{Sc}-specific binding matrix (eg. plasminogen, angiostatin or antibodies) can identify and concentrate PrP^{Sc} for further detection. This should be an ideal test to detect prion diseases such as BSE and CJD in symptom-free animals and humans and potentially clear the pathological prions from blood products.

Human Plasma Prion Separation and Concentration

There are currently no effective strategies for early diagnosis or therapeutic intervention of TSE disease. Although a few blood-screening test methods are in different stages of development, low amounts of infectious prion protein present early in the disease represents a challenge for established detection methods. Although multiplication of prions is one such strategy for easy

detection, a possible option would be if the prions can be concentrated prior to the use of detection tests.

Gradiflow™ is an unique membrane-based electrophoresis separation technology developed by Gradipore Limited, Australia that can use both the molecular characteristics of size and charge to purify individual components from biological samples. Several membrane-based electrophoresis apparatus were investigated as possible candidates for use with the present invention.

Size-based separation

Size-based separation of PrPc was carried out for 2 hours at 250V using Tris Borate buffer (20 mM Boric Acid, 45 mM Trizma Base, pH 9.0) as the running buffer. All cartridge set ups involved the use of two small-type restriction membranes designated a number 10 having a molecular mass cut-off of about 10 kDa and a separation membrane of varying size restrictions with designated numbers ranging from 100 to 1000 having a molecular mass cut-offs of about 100 kDa to 1000 kDa, respectively.

The results of initial experiments described above are shown in Table 1 and Figure 3

Table 1. Size-based separation of PrPc from normal human plasma.

Run	Cartridge	Starting material	Stream 1 (% of total starting PrP)	Stream 2 (% of total starting PrP)
1	10-100-10	Plasma	115	*
3	10-200-10	Plasma	80	10
5	10-800-10	Plasma	53	25
7	10-1000-10	Plasma	*	58

* – Below limit of detection

Figure 3 shows the results of a SDS-PAGE analysis of the electrophoresis run number 1 and 7. Lanes 1, 2, 3, 4 are S1 0 minutes, S1 120 minutes, S2 0

minutes, S2 120 minutes of run 1, respectively. Lanes 5, 6, 7, 8: S1 0 minutes, S1 120 minutes, S2 0 minutes, S2 120 minutes of run 7, respectively. From the this analysis of the electrophoretic runs, it was evident that low molecular weight proteins came through the smallest-type separation membrane designated 100 having a molecular mass cut-off of about 100 kDa, while the use of the large-type separation membrane having a molecular mass cut-off of about 1000 kDa resulted in the complete transfer of albumin and other high molecular weight proteins from the samples.

Charge-based separation

Charged-based separation of PrPc was carried out for 2 hours at 250V using a cartridge set up consisting of two small-type restriction membranes designated a number 10 having a molecular mass cut-off of about 10 kDa and a large-type separation membrane designated a number 1000 having a molecular mass cut-off of about 1000 kDa. Different buffers used were in the range pH 4.8 to pH 9.0. For all of the runs listed in Table 2, normal human plasma was used as the starting material and the source of PrPc.

Table 2. Charge-based separation using a LARGE-type separation membrane

Run	Buffer pH	Polarity	Stream 1 (% of total starting PrP)	Stream 2 (% of total starting PrP)
10	MES/□-Alanine pH 4.8	Reverse	67	*
11	MOPS/GABA pH 5	Forward	40.2	13.5
14	Hepes/Imidazole pH 6	Forward	53	10
B08	MOPS/□-Alanine pH 6.5	Forward	44.7	13.1
15	Hepes/Imidazole pH 7	Forward	19.5	39.2
16	Tris/Borate pH 8	Forward	56	10
B14	Tris/Borate pH 8.5	Forward	19.6	38.6
17	Tris/Borate pH 9	Forward	*	58

* – Below limit of detection

Charged-based separation of PrPc was carried out for 2 hours at 250V using a cartridge set up consisting of two small-type restriction membranes designated a number 10 and a Large-type separation membrane designated a number 1000. Different buffers used were in the range pH 4.6 to pH 9.0. For all

of the runs listed in the table, normal human plasma alone was used as the starting material and the source of PrPc.

Table 3. Charge-based separation using a SMALL-type separation membrane

Run	Buffer pH	Polarity	Stream 1 (% of total starting PrP)	Stream 2 (% of total starting PrP)
19	MES/B-alanine pH 4.8	Reverse	81%	*
20	MOPS/GABA pH 5	Reverse	82%	*
21	Hepes/Imidazole pH 6	Reverse	79%	*
22	Hepes/Imidazole pH 7	Reverse	74%	*
23	Hepes/Imidazole pH 6	Forward	73%	*
24	Hepes/Imidazole pH 7	Forward	68%	*
25	Tris/Borate pH 8	Forward	84%	*
26	Tris/Borate pH 9	Forward	80%	*

* – Below limit of detection

A two step separation process of PrPc was performed in order to achieve isolation of PrPc from the majority of normal human plasma proteins. First step separation (a charge-based separation) of PrPc was carried out in reverse polarity for 2 hours at 250V using MOPS/GABA buffer (80 mM MOPS, 20 mM

GABA, pH 5.0) as the running buffer. The anode was positioned adjacent stream 1 and the cathode positioned adjacent stream 2 (reverse polarity configuration). The plasma sample was placed in stream 1 and subjected to electrophoresis. The electrophoresis cartridge consisted of two small-type restriction membranes designated a number 10 having molecular mass cut-off of about 10 kDa and a large-type separation membrane designated a number 1000 having a molecular mass cut-off of about 1000 kDa positioned between the restriction membranes. Both high and low molecular weight proteins with pI's greater than 5.0 were moved from the sample in stream 1 to stream 2 and the partially purified PrPc remained in stream 1. The PrP fraction from the first step was then used as the starting material for the second step.

Second step separation (a size-based separation) of PrPc was carried out in forward polarity for 2 hours at 250V using Tris Borate buffer (20 mM Boric Acid, 45 mM Trizma Base, pH 9.0) as the running buffer. The cathode was positioned adjacent stream 1 and the anode positioned adjacent stream 2 (normal polarity configuration). The PrP fraction from the first step was placed in stream 1 and subjected to electrophoresis. The electrophoresis cartridge set up consisted of two small-type restriction membranes designated a number 10 having molecular mass cut-off of about 10 kDa and a small-type separation membrane designated a number 200 having a molecular mass cut-off of about 200 kDa. Low molecular weight proteins with pI less than 9.0 were removed to stream 2 while PrPc was fully retained in stream 2.

Two-step separation protocol resulted in removal of contaminant proteins with pI between 5 to 9 (90% Albumin, 80% IgG, 100% α1-proteinase inhibitor removed).

Results of the separation using Step 1 and Step 2 are shown in Figure 4.

Simultaneous separation and concentration of human plasma PrP

The electrophoresis cartridge set up of the second step of the two-step separation process (described above), was modified such that the upper restriction membrane which forms part of stream 1 was replaced with a polyvinyl-alcohol membrane (PVA) (endosmosis membrane) in order to achieve a

simultaneous separation and concentration of PrP. At the end of the separation process, a 3 fold increase in the concentration of PrP was achieved based on the quantitative DELFIA analysis. A variety of other endosmosis membranes can be used including cellulose triacetate (CTA).

Results of this separation are shown in Figure 5.

Bovine Prion Separation and Concentration

Figure 6 shows the results of simultaneous separation and concentration of the bovine prion protein (PrP) from bovine brain homogenate using membrane-based electrophoresis technology and a PVA membrane. Panel A shows SDS-PAGE analysis of the samples from the electrophoresis run. Panel B shows a Western blot of the samples from the electrophoresis run using anti-PrP R029 antibody (Prionics, Switzerland). $S1_0$ is Stream 1 at time 0 minutes, $S1_{180}$ is Stream 1 at time 180 minutes, $S2_0$ is Stream 2 at time 0 minutes, $S2_{180}$ is Stream 2 at time 180 minutes. Separation of PrP was carried out for 3 hours at 250V using a cartridge with a separation membrane of 200 kDa, and two restriction membranes of 5 kDa with Tris Borate buffer (20 mM Boric Acid, 45 mM Trizma Base, pH 9.0) being applied (Panel A, lanes 1-4), the conditions under which PrP^C remained in the upstream (Panel B, lanes 1-4). Simultaneous separation and concentration of PrP^C from bovine brain homogenate was achieved under the same buffer running conditions, but with a PVA membrane being used as the top restriction membrane in place of the 10 kDa restriction membrane (Panel B, lanes 5-8). There was a noticeable increase in the concentration of PrP, as detected in the increase of the intensity of the PrP band on the Western blot (panel B).

SUMMARY

There are currently no effective strategies for early diagnosis or therapeutic intervention of TSE disease. Although a few blood-screening test methods are in different stages of development, inadequate amounts of infectious prions (PrP^{Sc}) present early in the disease and therefore represents a challenge

for the currently established detection methods. One important requirement in developing a viable prion diagnostic device/kit is achieving successful PrP^{Sc} separation and concentration. A distinct advantage of membrane-based electrophoresis such as Gradiflow™ is that it can conduct a fast prion separation based on its size/charge with a small volume of biological samples. membrane-based electrophoresis has been shown by the present inventors to either separate or retain prion proteins from biological materials, thus allowing for the capture, partial purification and removal of prions. This exemplifies the potential of membrane-based electrophoresis as a suitable step for PrP^{Sc} separation and enrichment prior to assay. An aim of the present invention is to develop a rapid separation and extremely sensitive detection system for PrP^{Sc} in infectious biological samples. Ultimately, the aim is to develop a prion diagnostic test that allows detection of PrP^{Sc} in symptom-free infected animals and humans.

There are two major markets for a prion separation and detection device/kit. One is the diagnosis of BSE in living animals. The European Union (EU) has ordered that all cattle older than 30 months be tested before they can enter the food supply, creating a market for prion tests. Although BSE has never been found to occur naturally in sheep, Britain's Food Standards Agency urged a mass screening to determine whether BSE has spread to some of Britain's 40 million sheep in the fear that the presence of scrapie in up to 10,000 sheep a year could be "masking" the presence of BSE. That market could grow further if other countries follow Europe's lead. BSE is not a major issue in the United States at this time because Americans have been assured that no cases of BSE or vCJD have been confirmed. However, the United States Department of Agriculture (USDA) has scheduled surveillance testing programs and beef exports may come under testing requirements. Thus, a significant increase in demand for prion tests will be expected - 3.4 million cattle were slaughtered for food in US last year.

The potential health risk that the vCJD disease may be spreading through blood transfusions has created another major market for diagnosis of vCJD in humans. Safety measures have already been introduced over the past three years in UK - including the removal of white cells thought most likely to transmit vCJD. In addition, plasma is now being imported from the United States, but

further strategies will be essential to mitigate the unquantified risk. Potential donors are likely to be screened compulsorily before they can give blood. In UK, blood donor numbers were circa 2.2 million per annum in England and North Wales, with 440,000 per annum in Scotland and in Northern Ireland. In the United States alone, human blood is donated more than 12 million times a year. The market of prion blood-screening diagnostics will expand tremendously once the legislation is in place.

Testing for the presence of infectious PrP^{Sc} not only requires very sensitive and specific detection assay but also a separation system with capacity for automation and mass screening operation. Our product is defined as a separation device and a reagent kit including suitable cartridges and buffers. The separation device will be progressively developed into the following models:

Using normal bovine prion protein (PrP^{C}) as a surrogate for the abnormal form associated with TSE infectivity, conditions have been established for membrane-based electrophoresis to separate or retain prion protein from or in bovine brain tissue homogenates, thus allowing for the capture, purification and removal of prions. In addition, a simultaneous separation and concentration of prion protein from bovine brain homogenate was achieved by utilising electro-endosmosis (EEO) membrane.

To investigate the capability of membrane-based electrophoresis to remove prions during purification process of plasma products, a partitioning study was performed by spiking bovine prion protein into human plasma. Complete prion clearance of the original spike was achieved during the purification of human albumin, immunoglobulin and α1-proteinase inhibitor by membrane-based electrophoresis. The findings suggest that membrane-based electrophoresis has the potential to remove causative TSE agents during plasma fractionation, thus eliminating the risk of prion disease transmission.

Using human plasma as a starting material, size-based separation of normal prion protein was performed followed by a charge-based separation. The amount of prion protein in human plasma was quantified by a time-resolved dissociation-enhanced fluoroimmunoassay (DELFIA®). Results clearly indicate that membrane-based electrophoresis is able to purify and concentrate prion

protein from human plasma. Membrane-based electrophoresis has, therefore, the potential for not only abnormal prion protein separation but also enrichment prior to a suitable detection assay.

In addition, membrane-based electrophoresis has also provided a valuable insight into the physical characterisation of normal prion protein such as size and pI that has hitherto been unobtainable by other means due to the low quantities of prion in blood. Simultaneous isolation and concentration of abnormal prion protein from blood components by membrane-based electrophoresis is a suitable means for developing a diagnostic assay. Membrane-based electrophoresis, when combined with prion detection methods, can be used as a diagnostic tool for screening prion infectivity in humans and livestock.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

Claims:

1. A method for detecting prions in a sample, the method comprising:

(a) subjecting a sample suspected of containing prions to membrane-based electrophoresis to separate or concentrate at least some prions present in the sample; and

(b) detecting the presence of the separated and/or concentrated prions.

2. The method according to claim 1 wherein the sample is a biological sample selected from the group consisting of whole blood, plasma, serum, cell products, cell extracts, cerebrospinal fluid, tissue homogenates, urine, semen, and combinations thereof.

3. The method according to claim 1 wherein step (a) comprises:-

subjecting a sample suspected of containing prions to membrane-based electrophoresis to concentrate at least some prions into a smaller volume.

4. The method according to claim 1 wherein step (a) is carried out using an electrophoresis apparatus comprising:

(i) an electrolyte reservoir;

(ii) a first sample reservoir and a second sample reservoir;

(iii) a separation unit having a first electrolyte chamber in fluid connection with the electrolyte reservoir, a second electrolyte chamber in fluid connection with the electrolyte reservoir, a first sample chamber positioned between the first electrolyte chamber and the second electrolyte chamber, a second sample chamber positioned adjacent to the first sample chamber and between the first electrolyte chamber and the second electrolyte chamber, the first sample chamber being in fluid connection with the first sample reservoir, and the second sample chamber being in fluid connection with the second sample reservoir;

(iv) a first ion-permeable barrier positioned between the first sample chamber and the second sample chamber, the first ion-permeable barrier

prevents substantial convective mixing of contents of the first and second sample chambers;

(v) a second ion-permeable barrier positioned between the first electrolyte chamber and the first sample chamber, the second ion-permeable barrier prevents substantial convective mixing of contents of the first electrolyte chamber and the first sample chamber;

(vi) a third ion-permeable barrier positioned between the second sample chamber and the second electrolyte chamber, the third ion-permeable barrier prevents substantial convective mixing of contents of the second electrolyte chamber and the second sample chamber;

(vii) electrodes positioned in the first and second electrolyte chambers;

(viii) means for supplying electrolyte from the electrolyte reservoir to the first electrolyte chamber and the second electrolyte chamber; and

(ix) means for supplying sample or liquid from at least the first sample reservoir to the first sample chamber, or from the second sample reservoir to the second sample chamber.

5. The method according to claim 4 wherein the first, second and third ion-permeable barriers are membranes having a characteristic average pore size and pore size distributions.

6. The method according to claim 5 wherein the first ion-permeable membrane has a characteristic average pore size and pore size distribution greater than that of the second and third ion-permeable membranes.

7. The method according to claim 4 wherein the first ion-permeable barrier is an electrophoresis separation membrane formed from polyacrylamide.

8. The method according to claim 7 wherein the electrophoresis separation membrane has a molecular mass cut-off up to about 1500 kDa.

9. The method according to claim 4 wherein the second and third ion-permeable barriers are restriction membranes which are formed from

polyacrylamide and have a molecular mass cut-off less than the separation membrane.

10. The method according to claim 9 wherein the second and third ion-permeable barriers have a molecular mass cut-off from about 1 kDa to about 500 kDa.

11. The method according to claim 4 wherein at least one of the second or third ion-permeable barriers is capable of controlling substantial bulk movement of liquid under the influence of an electric field and is an inducible electro-endo-osmotic membrane.

12. The method according to claim 11 wherein the inducible electro-endo-osmotic membrane is a poly-vinyl alcohol membrane.

13. The method according to claim 4 wherein the first ion-permeable barrier is an electrophoresis membrane having a molecular mass cut-off of about 1000 kDa and the second and third ion-permeable barriers are restriction membranes having a molecular mass cut-off of about 10 kDa.

14. The method according to claim 4 wherein the first ion-permeable barrier is an electrophoresis membrane having a molecular mass cut-off of about 1000kDa, the second ion-permeable barrier is an inducible electro-endo-osmotic membrane, and the third ion-permeable barrier is a restriction membrane having a molecular mass cut-off of about 10 kDa.

15. The method according to claim 4 wherein the separation unit is provided as a removable cartridge or cassette housing the ion-permeable barriers.

16. The method according to claim 1 wherein step (b) is an immuno-detection assay.

17. The method according to claim 16 wherein the immuno-detection assay is an enzyme linked immunosorbant assay, Western blot, ELISA, time-resolved dissociation-enhanced fluoroimmunoassay (DELFIA) or radioimmunoassay.

18. The method according to claim 1 wherein step (b) is a prion capture system utilising a selective binding agent.

19. The method according to claim 18 wherein the selective binding agent is a biological agent which selectively binds prions or a chemical agent which selectively binds prions.

20. The method according to claim 19 wherein the selective agent preferentially binds infectious prions.

21. The method according to claim 19 wherein the biological agent is selected from the group consisting of protein, peptide or other small molecule, antibody, binding fragment of an antibody.

22. The method according to claim 21 wherein the biological agent is selecvted from the group consisting of plasminogen, angiostatin, fibrin, fibrinogen, and antibody.

23. The method according to claim 19 wherein the chemical agent is a heteropoly acid or salts thereof.

24. The method according to claim 23 wherein the heteropoly acid is phosphotungstic acid or salts thereof.

25. The method according to claim 1 wherein step (b) allows detection and identification of infectious prions.

26. The method according to claim 1 further comprising:

(c) characterising the detected prions.

26. The method according to claim 25 wherein the characterising is selected from the group consisting of solubility, three-dimensional structure, Circular Dichroism (CD), hydrophobicity, infectivity, and other physical and biochemical properties.

27. A method for detecting prions in a sample, the method comprising:

(a) subjecting a sample selected from the group consisting of whole blood, plasma, serum, cell products, cell extracts, cerebrospinal fluid, tissue homogenates, urine, semen, and combinations thereof suspected of containing prions to membrane-based electrophoresis in an electrophoresis apparatus to separate or concentrate at least some prions present in the sample; the membrane electrophoresis apparatus comprising (i) an electrolyte reservoir, (ii) a first sample reservoir and a second sample reservoir, (iii) a separation unit having a first electrolyte chamber in fluid connection with the electrolyte reservoir, a second electrolyte chamber in fluid connection with the electrolyte reservoir, a first sample chamber positioned between the first electrolyte chamber and the second electrolyte chamber, a second sample chamber positioned adjacent to the first sample chamber and between the first electrolyte chamber and the second electrolyte chamber, the first sample chamber being in fluid connection with the first sample reservoir, and the second sample chamber being in fluid connection with the second sample reservoir, (iv) a first ion-permeable membrane having a molecular mass cut-off of 100 kDa to 1000 kDa positioned between the first sample chamber and the second sample chamber; (v) a second ion-permeable membrane having a molecular mass cut-off of 10 kDa positioned between the first electrolyte chamber and the first sample chamber, (vi) a third

ion-permeable having a molecular mass cut-off of 10 kDa positioned between the second sample chamber and the second electrolyte chamber; (vii) electrodes positioned in the first and second electrolyte chambers, (viii) means for supplying electrolyte from the electrolyte reservoir to the first electrolyte chamber and the second electrolyte chamber, and (ix) means for supplying sample or liquid from at least the first sample reservoir to the first sample chamber, or from the second sample reservoir to the second sample chamber; and

(b) detecting the presence of the separated or concentrated prions by an immuno-detection assay selected from an enzyme linked immunosorbant assay, Western Blot, ELISA, time-resolved dissociation-enhanced fluoroimmunoassay (DELFIA) or radioimmunoassay.

28. A method for detecting prions in a sample, the method comprising:

(a) subjecting a sample selected from the group consisting of whole blood, plasma, serum, cell products, cell extracts, cerebrospinal fluid, tissue homogenates, urine, semen, and combinations thereof suspected of containing prions to membrane-based electrophoresis in an electrophoresis apparatus to separate or concentrate at least some prions present in the sample; the membrane electrophoresis apparatus comprising (i) an electrolyte reservoir, (ii) a first sample reservoir and a second sample reservoir, (iii) a separation unit having a first electrolyte chamber in fluid connection with the electrolyte reservoir, a second electrolyte chamber in fluid connection with the electrolyte reservoir, a first sample chamber positioned between the first electrolyte chamber and the second electrolyte chamber, a second sample chamber positioned adjacent to the first sample chamber and between the first electrolyte chamber and the second electrolyte chamber, the first sample chamber being in fluid connection with the first sample reservoir, and the second sample chamber being in fluid connection with the second sample reservoir, (iv) a first ion-permeable membrane having a molecular mass cut-off of 100 kDa to 1000 kDa positioned between the first sample chamber and the second sample chamber; (v) an inducible electro-endo-osmotic membrane formed from poly-vinyl alcohol positioned between the first electrolyte chamber and the first sample chamber, (vi) a second ion-

permeable having a molecular mass cut-off of 10 kDa positioned between the second sample chamber and the second electrolyte chamber; (vii) electrodes positioned in the first and second electrolyte chambers, (viii) means for supplying electrolyte from the electrolyte reservoir to the first electrolyte chamber and the second electrolyte chamber, and (ix) means for supplying sample or liquid from at least the first sample reservoir to the first sample chamber, or from the second sample reservoir to the second sample chamber; and

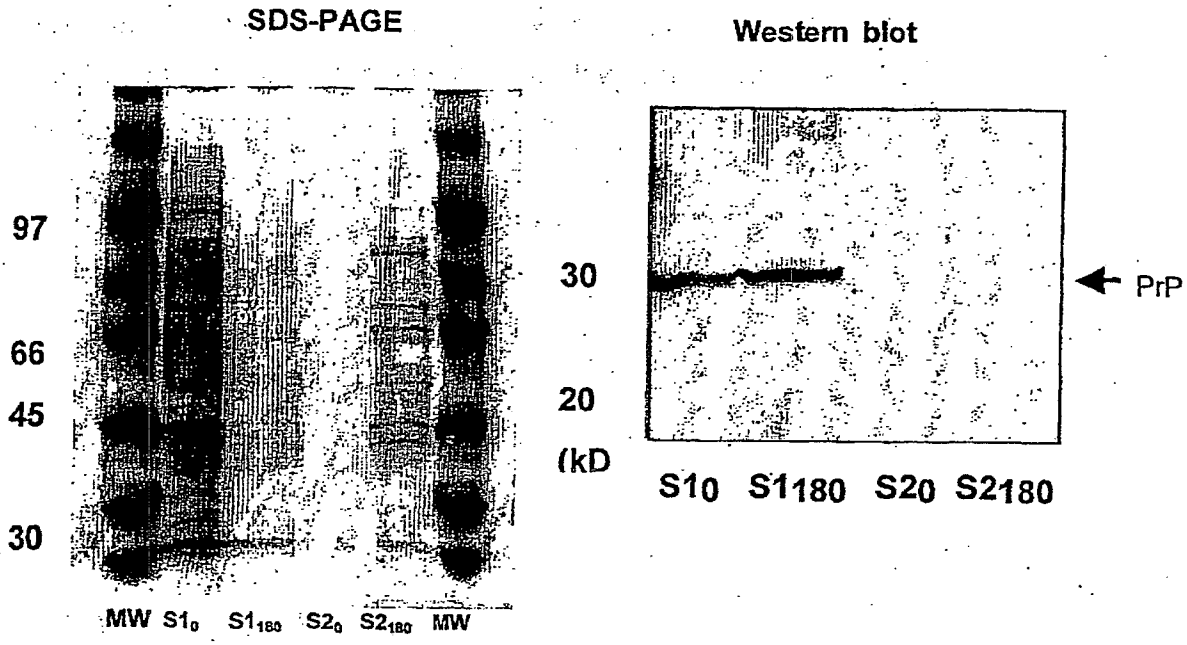
(b) detecting the presence of the separated or concentrated prions by an immuno-detection assay selected from an enzyme linked immunosorbant assay,Western Blot, ELISA, time-resolved dissociation-enhanced fluoroimmunoassay (DELFIA) or radioimmunoassay.

29. A kit for detecting prions in a sample, the kit comprising:-

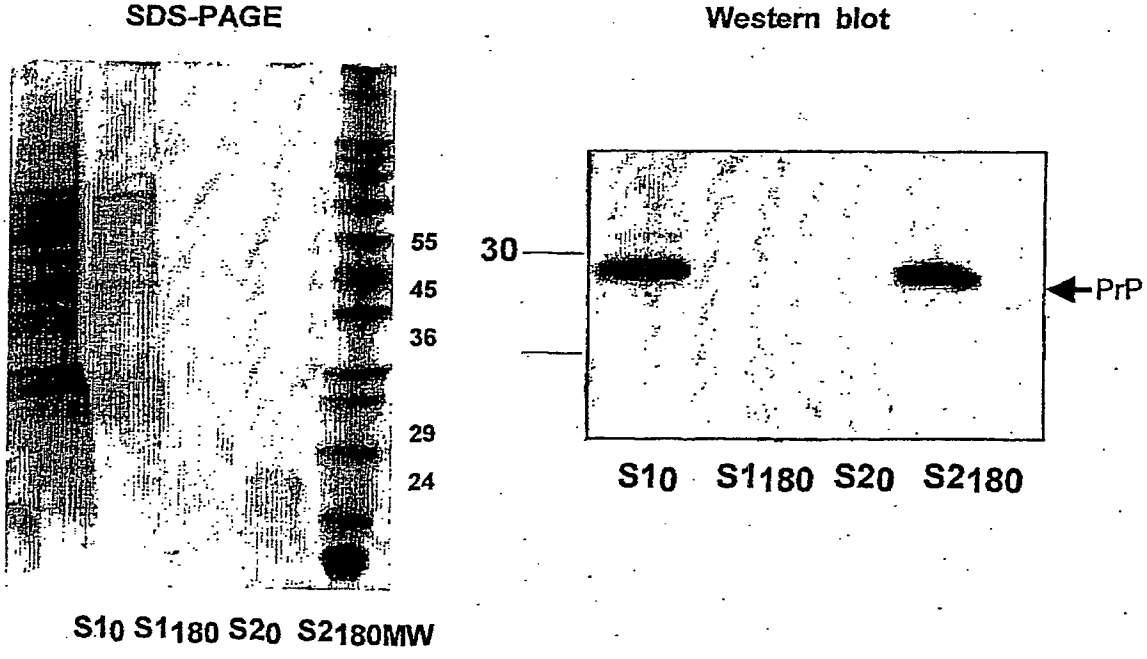
(a) a membrane-based electrophoresis apparatus suitable for separating or concentrating prions from a sample; and

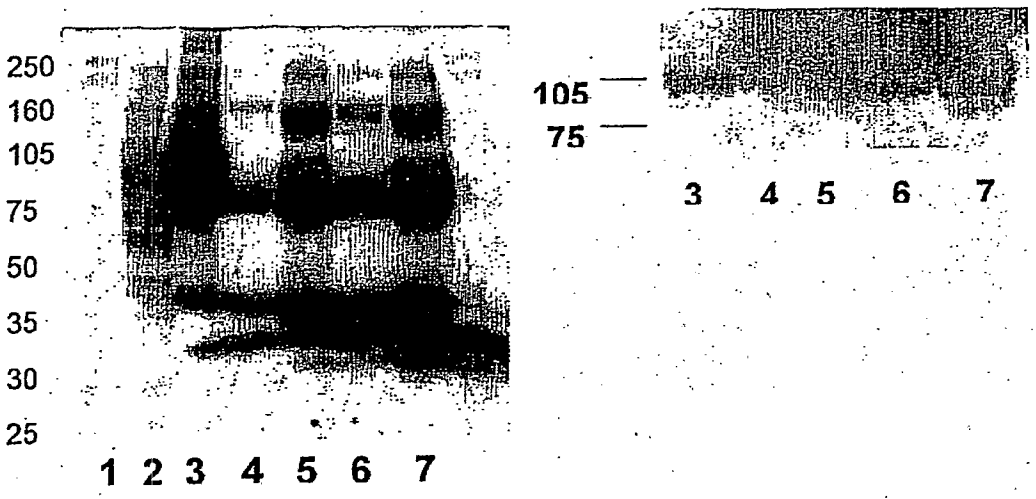
(b) means for detecting prions separated or concentrated by the electrophoresis apparatus.

Panal A



Panal B





Lane 1: MW

Lane 2: bovine brain tissue homogenates

Lane 3: human plasma

Lane 4: negative control-no antibody was added (protein A agarose)

Lane 5: negative control-no antibody was added (protein G agarose)

Lane 6: anti-PrP treated sample (protein A agarose)

Lane 7: anti-PrP treated sample (protein G agarose)

Figure 2

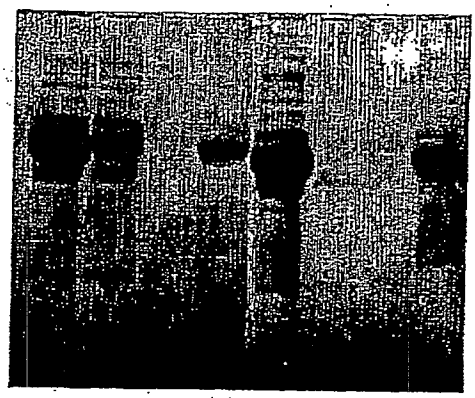


Figure 3

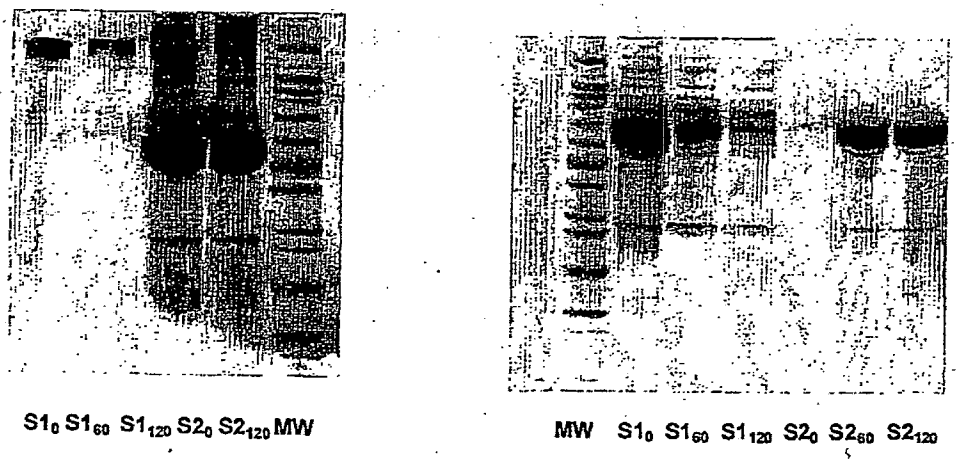
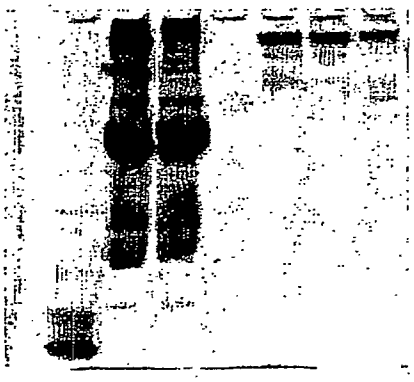


Figure 4

Step 1: charged-based



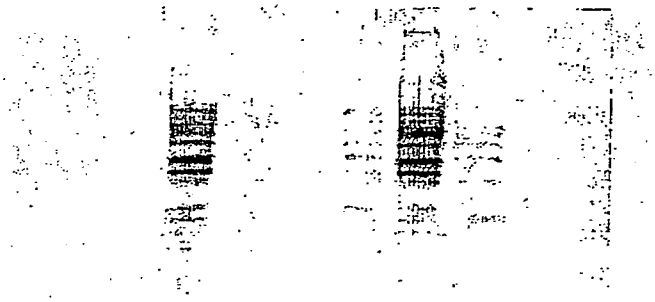
MW $S1_0$ $S1_{180}$ $S2_0$ $S2_{60}$ $S2_{120}$ $S2_{180}$

Step 2: size-based/concentration

MW $S1_0$ $S1_{180}$ $S2_0$ $S2_{60}$ $S2_{120}$ $S2_{180}$

Figure 5

A



$S1_0$ $S1_{180}$ $S2_0$ $S2_{180}$ $S1_0$ $S1_{180}$ $S2_0$ $S2_{180}$

B

$S1_0$ $S1_{1R0}$ $S2_0$ $S2_{1R0}$ $S1_0$ $S1_{1R0}$ $S2_0$ $S2_{1R0}$

Figure 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU02/00592

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: G01N 33/53, 33/68, 27/447; B01D 57/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

G01N 33/53, 27/26, 27/447; B01D 57/02

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPAT; Medline; CAPlus; JAPIO (prion; scrapie; PrP; membrane; electrophoresis; separat?; concentrat?; detect?

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	WO 01/78878 A1 (GRADIPORE LIMITED) 25 October 2001	
P, X	Whole document.	1-29
Y	Whole document.	1-29
	WO 00/40966 A1 (USA:THE SECRETARY OF AGRICULTURE) 13 July 2000	
Y	Whole document, particularly page 16 to page 18.	1-29
	WO 00/13776 A1 (GRADIPORE LIMITED) 16 March 2000	
X	Whole document.	1-29
Y	Whole document.	1-29

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
4 July 2002

Date of mailing of the international search report
16 JUL 2002

Name and mailing address of the ISA/AU

AUSTRALIAN PATENT OFFICE
PO BOX 200, WODEN ACT 2606, AUSTRALIA
E-mail address: pct@ipaustralia.gov.au
Facsimile No. (02) 6285 3929

Authorized officer

ALBERT S. J. YONG

Telephone No : (02) 6283 2160

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU02/00592

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01/10470 A1 (BAXTER AKTIENGESELLSCHAFT) 15 February 2001 Whole document.	1-3, 16-26
X	US 5,039,386 A (Margolis) 13 August 1991 Whole document.	1-3, 16-26, 29
X	WO 00/38743 A1 (GRADIPORE LIMITED) 6 July 2000 Whole document, particularly page 9 line 13 to page 11 line 9.	1-29
X	Horvath, Z.S. et al. (1994) Electrophoresis, vol. 15, pp 968-971 Multifunctional apparatus for electrokinetic processing of proteins. Whole document.	1-29

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/AU02/00592

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
US	5039386	AU	16280/88	EP	352286	WO	8807406
WO	200013776	AU	60689/99	EP	1128896	US	6328869
		US	2002023842				
WO	200040966	AU	200026043	BR	200007274	CZ	20012469
		EP	1147415	NO	20013193	US	6150172
WO	200110470	AU	200068386	US	6270672		
WO	200178878	AU	20006973	AU	200150172	US	2001052462
WO	200038743	AU	200025266	EP	1140214		

END OF ANNEX